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DIRECT ORGANOGENESIS OF *KALANCHOE TOMENTOSA* (CRASSULACEAE) FROM SHOOT-TIPS

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Abstract

An efficient and cost effective protocol for direct regeneration of *Kalanchoe tomentosa*, a valuable medicinal and ornamental plant, is presented here. Multiplication and growth responses of *Kalanchoe tomentosa* towards BAP (6-Benzyle amino purine), NAA (α- Naphthalene acetic acid) and a hormone free control MS medium was evaluated. The effects of BAP and NAA either singly or in combinations, to accelerate the organogenesis of *K. tomentosa* were examined. The cultures were grown for 28 days at 22 ± 2°C under 16 hours photoperiod with the light intensities of 3500 lux. Maximum number of shoots, length of shoots, number of leaves, number of roots and number of plants were obtained on a hormone free MS based medium (control), suggesting that there is a little role of plant growth regulators in the *In vitro* development, multiplication and organogenesis of *Kalanchoe tomentosa*. More than 95% of the well rooted plantlets were established *ex vitro* in green house which were phenotypically similar to their mother plants. The 1:1 ratio of sand and grinded charcoal proved best as a potting mix for the hardening of plants.

Introduction

Kalanchoe tomentosa the family *Crassulaceae* is a beautiful, perennial and succulent plant with dense white hairs-like covering. It is commonly known as Panda Plant and native to Madagascar. Genus *Kalanchoe* consists of about 130 species of annual and perennial shrubs, climbers and small trees. They usually occur in semi-desert or shady areas of Saudi Arabia, Yemen, Central Africa, Madagascar, Asia, Australia and tropical America. Usually, it is cultivated as garden ornamental in rock and sand gardens with a medium humidity (Brito *et al*., 1993). Apart from its ornamental value, *Kalanchoe* is also very well known as a medicinal plant and very famous for its antimicrobial, anti-inflamatory, antidiabetic, anti leishmanial and antitumor activity (Tadeg *et al*., 2005, Pal & Chandhuri, 1990, Supratman *et al*., 2001, Akinpelu, 2000, Santos, *et al*, 2003). The aqueous extract of *Kalanchoe pinnatum* also shows antidiabetic effect (Ojewole, 2005). Due to its enormous medicinal importance, the large scale production of this plant is the need of time.

Since, it is a slow growing plant therefore it is extremely necessary to develop a tissue culture system for quick production of plant for commercial and medicinal purposes. Although the micropropagation of medicinal plants has been studied extensively, reports on the production of secondary metabolites in *In vitro* cultures have not been much studied. Because of its medicinal importance and potential to produce value added secondary metabolites in tissue culture, it is of great interest to develop biotechnological methods to improve the production of this plant *In vitro*.

In the present investigation, attempts were made to reduce duration of micropropagation process using shoot tip as an explant source. On the other hand, the factors like plant growth regulators, with other physical parameters essential for plant regeneration, were also evaluated. This is the first report on direct organogenesis and establishment of complete plant from shoot tip of *Kalanchoe tomentosa*.

Table 1. Direct organogenesis from the shoot tip of *Kalanchoe tomentosa*.

Media code	BAP (mg/L)	NAA (mg/L)	Average number				
			Shoots	Length of shoots*	Leaves	Roots	Plants
C1	0.1	0	1.44±0.72	1.42±0.40	4.77±2.58	1.55±0.72	3.0±0.91
C2	0.2	0	1.22±0.66	1.37±0.31	5.22±4.96	1.11±0.33	3.1±0.54
C3	0.4	0	1.44±0.72	1.61±0.31	4.88±2.02	1.22±0.44	3.0±0.68
C4	0.8	0	1.11±0.33	1.43±0.12	6.11±2.47	1.11±0.33	2.6±0.45
C5	1.0	0	1.11±0.33	1.53±0.32	3.66±2.23	3.66±2.23	2.6±1.04
C6	0.1	0.1	1.22±0.66	1.46±0.20	3.77±2.48	1.44±1.01	3.2±1.12
C7	0.2	0.1	1.11±0.33	1.48±0.63	6.11±5.11	1.11±0.33	2.6±1.11
C8	0.4	0.1	1.11±0.33	1.37±0.40	3.33±1.65	1.33±1.00	2.6±0.65
C9	0.8	0.1	1.11±0.33	1.55±0.46	3.44±1.50	1.11±0.33	2.6±0.74
C10	1.0	0.1	1.11±0.33	1.34±0.44	3.77±2.27	1.33±0.50	3.3±0.24
C11	0.1	0.5	1.22±0.44	1.18±0.19	3.55±1.87	1.33±0.50	2.6±0.99
C12	0.2	0.5	1.11±0.33	1.71±0.60	5.00±1.93	1.33±0.50	2.6±1.01
C13	0.4	0.5	1.33±0.70	1.45±0.61	5.00±1.73	1.33±0.50	2.6±1.30
C14	0.8	0.5	1.33±0.50	1.64±0.42	4.22±2.58	1.22±0.44	2.6±0.85
C15	1.0	0.5	1.11±0.33	1.25±0.18	6.88±2.89	1.33±0.50	3.1±0.73
CC	0.0	0.0	1.66±1.00	1.77±0.38	7.66±4.77	6.55±3.39	3.4±0.52

Values represent Means ± SD (Standard Deviation).
* Length of shoots was measured in Inches.

Materials and Method

All the plant material was collected from the nurseries of ICCS, H.E.J. Research Institute of Chemistry. Shoot-tips were used as an explant. After isolation the shoot tips were washed with running tap water to remove all the sand and dust particles. Sterilization of the shoot tip was carried out by immersing them in the 0.1% mercuric chloride solution. One or two drops of Tween-20 were also added to reduce the surface tension. The solution with the shoot tips was agitated for 3-4 minutes. This was followed by three washes with autoclaved distilled water for 5 minutes each to remove traces of mercuric chloride.

After sterilization, the explants were further prepared by removing older leaves from the stalk. The explants were trimmed off to remove the dead portions which were in direct contact with the sterilant. After removing the dead portions, an equal size of explants were placed in jars containing various media as described Table 1. After transfer (one explant in each jar), all the jars were labelled and placed in growth room for 28 days at 22±2°C under fluorescent lights of 3500 lux for 16 hours. Three replications of each treatment were made and data were recorded after every week.

The initiated explants were transferred onto different shoot multiplication media to evaluate the effects of BAP and NAA (Table 1). After four weeks of transfer, the data on average number of shoots, length of shoots, number of leaves, number of roots and number of plants were collected and analyzed. Average increase in number and length of plants were also evaluated (Fig. 1B). Data was presented as means ± standard deviations in Table 1. The same cytokinin and auxin combinations were used to study their effect on root induction (Fig. 1C). Micropropagated plantlets were transferred to all the combinations and data were recorded after every week up to four weeks. Table 1 shows means ± SD of number of roots induced.

Plantlets that have been sufficiently rooted (three to four roots) *In vitro,* were removed from the culture vessel and washed thoroughly with water. Initially four different potting mixes were used for the acclimatization study. The four potting mixes comprised of 100 % sand, 50% sand with 50% grinded charcoal, 50% sand with 50%

coconut husk and 50% sand with 50% farm yard manure (v/v) (Fig. 1D). Data were collected and the survival rates using different potting mixes were calculated after six weeks.

Fig. 1. A- Initiation from shoot tip. B- Shoot multiplication. C- Rooting stage (arrows shows emerging roots). D- Acclimatization, arrow pointed the best response on 1:1 ratio of sand and charcoal (after 6 weeks).

Results and Discussion

The presented study was targeted to establish an efficient, fast and reliable protocol for the direct organogenesis of the *Kalanchoe tomentosa* plant from the shoot tips.

The sterilization procedure proved very effective since more than 95% culture proved clean and only 3% of the total initiated plants were contaminated by bacterial contamination. The explant was successfully initiated on simple MS media and shoot proliferation and multiplication was observed within 8-10 days. After 14 days, the initiated plants, which sufficiently developed, were transferred to all the experimental media with a control i.e. simple MS media without any plant growth regulators (Table 1). Previous studies on the micropropagation of *Kalanchoe tomentosa* reported the use of

different plant growth regulators like IAA, 2, 4-D, NAA and TDZ (Frello *et al*., 2002, Dickens & Staden, 1990, Ioannou & Ioannou, 1992). In this study maximum number and length of shoots, number of leaves and number of roots with number of plants were achieved on simple MS media and thus our protocol was much effective in terms of over all cost of the tissue culture. Our results, would suggest that the maximum number of shoots (1.66±1.00), length of shoots (1.77±0.38) along with maximum number of leaves (7.66±4.77), can be achieved on simple MS media devoid of any hormones. Maximum number of plants were also achieved on the same media. These results might be due to the fact that *Kalanchoe tomentosa* produced sufficient amounts of both cytokinins and auxins endogenously as the addition of BAP and NAA to the growth media didn't significantly improve the shoot proliferation.

Kalanchoe tomentosa was found to be very rapidly growing plant as maximum shoot proliferation was achieved just after 4 weeks of culture and within a single jar there were at least 15-20 *in vitro* grown plantlets. It was also observed that orientation of an explant was also critical as enhanced shoot multiplication was seen when the explants were placed horizontally.

The *In vitro* roots induction was also successfully achieved on the same simple MS media and on average 6.55±3.39 roots were grown from each plantlet. The rooted plants were acclimatized *ex vitro* in the green house. The *In vitro* grown plants performed well under field conditions and they were morphologically identical to the mother plants. Four different potting mixes were used (100 % sand, 50% sand with 50% grinded charcoal, 50% sand with 50% coconut husk and 50% sand with 50% farm yard manure v/v) to optimize the best for the hardening of the plants. The potting mix comprised of 50% sand with 50% grinded charcoal proved the best (data not presented) for the acclimatization of the *in vitro* grown plantlets with the survival rate of 96%.

The results of the present study suggests that the *Kalanchoe tomentosa* plant can be sufficiently grown on the simple MS medium without any supplementation of auxins and cytokinins. The same medium is also best suited for the induction of roots from the *In vitro* grown plantlets. The 1:1 ratio of sand and grinded charcoal was found optimum for the hardening of the plants. The optimized protocol is fast, reliable and capable of producing large number of plants in a short period of time.

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